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Figure 9

Radio-Immunometric (two-site) assay using antibody B152 as capture and B207 as radiolabeled detection reagent. Binding curves are shown for competitors, various competitor as detailed in methods and results. Each panel represents a separate assay in which all ligands were introduced in the same assay. Points were connected by straight lines although regression analysis (4 parameter logistic) indicated excellent fit to logistic or sigmoidal curve shape model. Panel A and B represent two distinct assays with similar results. It is clear that this assay has greatest recognition of the nicked, choriocarcinoma hCG immunogen which is hyperglycosylated and binds similarly to nicked and non-nicked forms of hCG which contain the usual quantities of sugars. Reagent M1A is missing most of its beta COOH-terminal region, supporting a role of this region in the binding site of B152 (see panel B and discussion in text).

Figure 10

Enzymic-Immunometric (two-site) assay using antibody B152 as capture and peroxidase-labeled B4001 as detection antibody. A linear-linear plot of molar quantities of ligand added is plotted versus absorbance at 492nm which is the response factor from the peroxidase detection system. Eight different hormone forms were measured as ligands within the same assay as indicated in the legend and described in ~~figure 15 and 18~~ table 5 and 8. The two hyperglycosylated choriocarcinoma-derived hCG

isoforms are both the most potent ligands (Table IV). Potency correlated well with hyperglycosylation of ligand (see ~~Figures 15 and 18~~ Tables 5 and 8 and text).

Figure 11

Characteristics of the Reagents Used to Define Antibody Specificity. The peptide and carbohydrate structures of the reagents used were determined earlier (26). The % nicked β-subunit refers to the proportion of molecules with cleavages (missing peptide bonds) in the region β43 to β48. The % tetrasaccharide core is the proportion of O-linked oligosaccharides with tetrasaccharide (vs. disaccharide) core structure, and the % sialic acid, is the proportion of O-linked structures with antennae terminated by sialic acid residues. The proportion of triantennary N-linked oligosaccharides on β-subunit is given, as is the corresponding % sialic acid.

[a].% sialic acid residues per sugar chain, N-linked on β.

[b].% sialic acid residues per sugar chain, O-linked on β.

[c] The "CR" series of hCG reference preparations were made at Columbia University and were distributed internationally as reference materials for purified hCG. CR 119 is also known as the 3rd international immunoassay reference preparation for hCG.

[d] ND is not done; NA is not applicable to that reagent.

[e] Less than 15% of the beta COOH-terminal region is present on this preparation.

Figure 12

Affinity Constants[a] Determined by Liquid Phase Competition Assays Using C5 as Tracer Ligand.

[a]Ka as L/M

[b]hCG CR 127 is an NIH-distributed hCG reference preparation produced at Columbia University.

Figure 13

Matrices of data for binding characteristics of different pairs of detection antibodies using B151 or B152 as capture antibody.

A. Relative Cross-Reactivities of Two Site Assay Using B151 as Capture Antibody

[a] labeled detection antibodies

[b<] out of low range detection

[c] this particular assay format was applied in O'Connor et al (25).

B. Relative Cross-Reactivities of Two Site Assay Using B152 as Capture Antibody

The molar quantity of ligand required to produce binding equal to 50% of the maximum binding achieved by C5 was determined. Cross-reactivity shown in this figure as a percentage is calculated by dividing the molar quantity of the standard by the molar quantity of the other ligand at 50% maximum binding dose.

[a] labeled detection antibodies

[b] maximum binding represents the total quantity of radiolabeled detection antibody which can bind to the plate in the system described.

[c] out of low range detection

[d] this particular assay format was applied in O'Connor et al (25).

Figure 14

Immunoreactivity of antigens in the B152 immunoradiometric assay. The dose-response curves used to provide data for this figure are shown in ~~figure 17~~ table 7. Each curve was fitted with 4-5 points. Slope and coefficient of determination (R^2) were determined using a non-linear regression algorithm. Slopes were used as an indicator of antigen potency. Relative potency was estimated as the slope of antigens relative to the slope of C5 Choriocarcarcinoma hCG (the immunogen).

[a] Slope are from ~~figure 17~~ table 7 as calculated in Sigmaplot 4.01 by linear regression analysis. Units of slope are pmole/ml absorbance at 492nm.